BRAIN FREE AMINO ACID POOL IN RATS DIFFERING IN PREFERENCE FOR ETHANOL

S. Yu. Ostrovskii and K. Kiianmaa

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Sensitivity to alcohol and ability to utilize it to a greater or lesser degree are ultimately manifested as the so-called tolerance to ethanol, which plays an essential role in predisposition to alcoholism and in the pathogenesis of alcoholism itself [12]. Since amino acids are involved in the mechanisms of detoxication of the alcohol oxidation product acetaldehyde [8], and since they themselves are neurotransmitters or their precursors [6], the study of their brain levels in connection with elucidation of mechanisms of tolerance rests on a sufficiently firm basis.

EXPERIMENTAL METHOD

Experiments were carried out jointly with the research laboratories of the Finnish State Alcohol Company (Alko Limited), Helsinki, on inbred rats of lines resistant (AT) and sensitive (ANT) to the action of ethanol. The animals (seven in a group) were kept on a standard synthetic diet. After decapitation the brain was quickly removed and divided into the right and left hemispheres, cerebellum, and brain stem, which were immediately frozen in liquid nitrogen, and then homogenized with 3% sulfosalicylic acid. The content of free amino acids was determined [1] in the supernatants on a T-339 automatic amino acid analyzer (Czechoslovakia). The results were assessed by calculating the significance of differences between the mean values and coefficients of correlation on a "Mera-Kamak 125/SM4A" computer [4].

EXPERIMENTAL RESULTS

The distribution of the amino acids and of some of their derivatives among the brain regions studied in animals differing in preference for ethanol was revealed primarily as marked asymmetry between the left and right hemispheres (Table 1). In the first, concentrations of Ser, Gln, Gly, Cys, Phe, Lys, and His were lower in ANT, whereas in the second there was no difference, apart from an increased content of Thr. No difference in the content of free amino acids was found in the cerebellum and brain stem of the animals compared. The particularly high sensitivity of the brain to alcohol is already sufficiently well known [13], and for that reason the characteristics of the free amino acid distribution which we found in the right and left hemispheres call for very detailed further examination. It is remarkable that the differences we found in the amino acid levels in the left hemisphere of the groups of animals compared correspond almost perfectly to the notion of two mechanisms of tolerance to ethanol: neurogenic [12] and metabolic [7]. In fact, it may be postulated that in ANT rats systems controlled by the inhibitory amino acid Gly function in a unique manner, and the capacity of these animals to carry out adaptive catecholamine synthesis (less Phe) is limited, and systems indirectly involved in the maintenance of acetylcholine synthesis (Ser, Ala) are evidently defective also. Second, the metabolic pathway of tolerance, in the view of most research workers, is linked with processing or inactivation of the toxic oxidation product of ethanol, acetaldehyde [8]. The content of amino acids interacting with acetaldehyde [2, 3] is appreciably lower in the left hemisphere of ANT rats.

Institute of Biochemistry, Academy of Sciences of the Belorussian SSR, Grodno. Alko Research Laboratories, Helsinki, Finland. (Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 264-266, March, 1990. Original article submitted December 31, 1988.

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TABLE 1. Differences in Content of Free Amino Acids (nmoles/g) in Brain of AT and ANT Rats

Amino acids	Right hemisphere		Left hemisphere	
	AT	ANT	AT	ANT
Thr	103±22	206±43*	116±14	92±8
Ser	864±158	1107±272	808±97	540±50*
Gln	579±64	841±198	624±67	447±55*
Gly	311±24	338±59	330±18	222±12*
Ala	272±41	318±55	305±27	232±15*
Cys	86±26	61±11	96±11	57±15*
Phe	85±18	123±22	163±22	85±7*
Lys	1379±121	1484±188	1536±68	1051±87*
His	192±33	185±39	291±34	183±26*

Legend. $p < 0.05$-0.001.

TABLE 2. List of Within-Group Differences ($p < 0.05$) in Content of Amino Acids between Parts of the Brain of AT and ANT Rats

Amino acids	AT	ANT
Thr	Absent	RL
Glu	RC, LC	RC
Gln	RC, LC, SC	Absent
Ala	LC, SC	«—»
Cys	LC	«—»
Ile	RS, LS	«—»
Phe	LR, LC	«—»
GABA	RC, SC	«—»
Lys	LC	«—»
His	RC, RS, RL	«—»
Tau	LC	«—»

Legend. R) Right hemisphere, L) Left hemisphere, C) cerebellum, S) brain stem.

Some more general parameters for the left hemisphere deserve further examination. For instance, the fall of the Gly level in this part of the brain was partially compensated by an increase in the content of other inhibitory amino acids, and for that reason the sum of GABA + Gly + Tau in the ANT rats was appreciably higher (4.5 ± 0.5 and 6.8 ± 1.3 μmoles/g, $p < 0.1$) than in AT animals. Tolerance to ethanol may be characterized by different parameters [11, 12], and the data given above provide a good explanation of differences in the response of the ANT rats to alcohol. The reduced capacity of the ANT rats to utilize amino acids for catecholamine synthesis must have the same consequences: the sum of the concentrations of the corresponding precursors (Tyr + Phe) in the left hemisphere of the ANT animals was appreciably lower (0.32 ± 0.05 and 0.19 ± 0.02 μmole/g, $p < 0.03$). Finally, if the whole amino acid pool is regarded as a reserve of adaptive reactions, i.e., of the ability of the animal to withstand the action of alcohol, then by the same approach the ANT rats have the advantage over AT rats. In the former, the content of essential (1.80 ± 0.05 and 2.61 ± 0.15 mole/g, $p < 0.001$) and nonessential (5.35 ± 0.59 and 7.00 ± 0.73, $p < 0.1$) amino acids in the left hemisphere is smaller. It can be concluded from the data given above that differences in the behavior of ANT rats after taking ethanol are connected primarily with the character of its supply of the various amino acids.

Since the behavioral reactions are an integral response of the CNS as a whole, we must examine the distribution of amino acids between different parts of the brain within each group of animals (Table 2). We shall examine only those parameters for which significant ($p < 0.05$) differences were recorded in at least one pair of brain regions compared in the same variety of animals. It is perfectly evident from the data in Table 2 that the mosaic pattern of distribution of different amino acids is many times higher in the brain of AT rats. Relations of the cerebellum with other parts of the brain studied differ particularly in this respect. Purely on the basis of this information it can easily be accepted that the powers of adaptation of animals of the line highly tolerant to ethanol ought to be superior, for integrative connections between different parts of the brain are responsible for organized general responses such as sleep and motor activity [5].

TABLE 3. Coefficients of Correlation (r) of GABA Level with Its Precursors and Functionally Connected Amino Acids

Pairs compared	Right hemisphere		Left hemisphere		Cerebellum		Brain stem	
	AT	ANT	AT	ANT	AT	ANT	AT	ANT
GABA – Glu	0,83*	0,21	0,57	0,98*	0,03	0,90*	0,77*	0,49
GABA – Gln	0,85*	0,11	0,83*	0,98*	—0,55	0,92*	0,84*	0,74*
GABA – Asp	0,81*	0,22	0,87*	0,97*	0,52	0,87*	0,84*	0,96*
GABA – Gly	0,85*	0,26	—0,06	1,00*	0,15	0,72*	0,94*	0,30

Legend. *p < 0.05-0.01.

Specific functional analysis of relations between individual amino acids by correlation analysis reveals additional differences between AT and ANT rats. For instance, coordinated changes in the levels of two inhibitory amino acids (Gly and GABA) are found in highly tolerant rats only in the left hemisphere and brain stem (r = 0.85 and 0.94 respectively), but not in ANT animals (r = 0.26 and 0.30 respectively). Opposite relations are characteristic of the same varieties of rats, but for the right hemisphere (r = —0.06 and 1.00) or the cerebellum (r = 0.15 and 0.72). GABA system in different parts of the brain in individuals compared, judging by the levels of correlation discovered (Table 3), is very heterogeneous.

Metabolism of the amino acids with the formation of ammonia also takes place in AT and ANT animals with the use of both similar and very different mechanisms in different parts of the brain. For instance, the direct involvement of Gly in ammonia production [9] is negligible (to judge from the fact that the coefficients of correlation are not significant) in the left hemisphere and brain stem of both groups of animals, and it plays an essential role only in the cerebellum (r = 0.90) and right hemisphere (r = 0.95) only of ANT, but not of AT (r = 0.14 and 0.32 respectively) lines of rats. Binding of ammonia by amidation of Glu [10] in all parts of the brain (according to coefficients of correlation for Glu—Gln) takes place very actively in rats of both varieties, whereas reductive amination of ketoglutarate, characterized by the Glu—NH_3 ratio, evidently takes place similarly (r = 0.92 and 0.94, p < 0.01), only in the right cerebral hemisphere. In the cerebellum this process is possible only in ANT animals, and can hardly be active in the left hemisphere and brain stem (r between —0.07 and 0.31, p > 0.5) of the two groups of rats compared. Finally, participation of the branched amino acid Ile in the mechanisms of Gln formation [14, 15] differs in the brain stem and cerebellum compared with both hemisphergin AT and ANT rats. In the first two brain regions of the AT strain no correlation is found between the Ile and Gly levels, whereas in ANT the corresponding coefficients are positive and highly significant (p < 0.05-0.02). In the left hemispheres of AT and ANT rats no correlations could be found between Ile and Gln levels, whereas in the right hemisphere only of AT rats was significant correlation observed (r = 0.75, p < 0.05) between the two parameters.

Thus lines of rats genetically differing in their tolerance to ethanol (AT, ANT) differ significantly from one another principally in the higher content of Ser, Gln, Gly, Cys, Phe, Lys, and His in the left cerebral hemisphere of the AT rats. The differences thus discovered may be important in the mechanisms of metabolic and neurogenic tolerance to ethanol. Moreover the two different strains of animals differ in relations between the amino acid pools of the right and left hemispheres, cerebellum, and brain stem in animals of the same strain.

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